THE CYTOLOGICAL MECHANISM OF CROSSING OVER

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With plate 44, one text figure and 11 diagrams

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INTRODUCTION

Two theories concerning the mechanism of crossing over have recently been advanced. The first theory is essentially the "partial chiasmatypy" hypothesis of Janssen's (1924), which is based on the assumption that chiasmata are caused by crossing over between two of the four chromatids. This theory has been sponsored by Belling (1929, 1931, a. b.), Darlington (1930, 1931) and Maeda (1930). The other theory of crossing over is based on the assumption that chiasmata do not represent crossovers, but are caused by an alternate opening out of sister and non-sister chromatids at diplotene (Weinrich 1916; Robertson 1916; Wilson 1925; Seiler 1926; McClung 1927; Belar 1928). A crossover occurs only when two chromatids break at a chiasma (Sax 1930).

An analysis of chromosome behavior at meiosis and an analysis of crossover types in the attached X chromosomes in *Drosophila melanogaster* should afford some critical evidence for a comparison of the two theories of the cytological mechanism of crossing over. This work is based on a cytological study of the chromosomes of *Callisia repens*, a survey of chiasma frequency in other plant genera, and an analysis of some of the genetic evidence obtained from *Drosophila*.

CHROMOSOME BEHAVIOR IN CALLISIA REPENS

In *Callisia repens* it is possible to follow the behavior of the chromosomes at all stages from pachytene to metaphase. This

material was collected and preparations made at the Harvard Botanic Garden, Soledad, Cuba. The writer is indebted to Professor Oakes Ames, Supervisor of the Arnold Arboretum and the Harvard Botanic Garden, for making possible the trip to Cuba.

Callisia repens has six pairs of large chromosomes. In two pairs of chromosomes the spindle fiber attachment points are more or less median while the other four pairs have nearly terminal fiber attachments. The twelve somatic chromosomes are shown in text-figure 1. The chromosome in the middle of the metaphase plate has one

FIG. 1. CHROMOSOMES OF CALLISIA REPENS

arm oriented at right angles to the plane of the other chromosomes. The sister chromatids in many of the chromosomes are clearly separated and show little twisting about each other. The limited number of twists or half twists in the somatic chromosomes is of special interest in connection with the theories of crossing over.

The study of meiotic chromosomes is based on smear preparations of pollen mother cells fixed in Navaschin's solution and stained with crystal violet iodine. The figures in the plate were drawn at a magnification of 3100 diameters and reduced about one-fourth in reproduction.

During the early pachytene stage the spireme is so compact that individual chromosomes cannot be identified. A small nucleolus is always present and is almost invariably at the periphery of the spireme and in contact with a free end of a chromosome thread. As the spireme opens up it is evident that it consists of six long interlooped chromosomes (Plate 44, fig. 1). The end of one of the shorter chromosomes is invariably in contact with the nucleolus. At a somewhat later stage the six bivalent chromosomes, still paired throughout their length, can be easily recognized. This stage is shown in figure 2. The two long bivalents are numbered 2 and 5. One of the short chromosomes, number 3, appears to have started

opening out into the typical nodes and internodes of the diplotene stage. The nodes and internodes at this stage appear to be much more numerous than at late diplotene.

The chromosomes shown in figure 3 have in most cases opened out into the diplotene loops, although parts of several chromosomes appear to have remained closely paired as they were at the typical pachytene stage. The nucleolus shows considerable disintegration at this stage and usually the side away from the chromosome attachment disappears first. The nucleolus is attached to one of the shorter chromosomes in all cases and it seems very probable that the same chromosome is always associated with the nucleolus.

When all of the chromosomes have reached the diplotene stage the number of nodes per chromosome ranged from two to more than five. It is not possible to recognize the individual chromatids in this material so that it is impossible to be sure that all of the nodes are chiasmata, but judging from the association of chromatids in *Orthoptera* chromosomes and at later stages in plant species it seems probable that most of them are chiasmata. The total number of nodes or chiasmata at diplotene is about twenty-five or an average of somewhat more than four per bivalent (Fig. 4.)

At early diakinesis the chromosomes become much thicker and shorter. An intermediate stage is shown in figure 5. The number of nodes or chiasmata is reduced to a total of about ten. The two long chromosomes seem to show some evidence of fiber constriction points but for the most part these constrictions do not show. Later stages of diakinesis are shown in figures 6 and 7. The total number of nodes is now at a minimum and no further reduction takes place before the chromosomes are separated at the first meiotic division.

The individual chromatids are not distinguishable even at the first metaphase but the configuration of the bivalents shows the number of attachment points. The chromonemata are distinctly coiled in some cases, but are not sufficiently clear for a critical study of the association of chromatids (Fig. 8). At telophase two of the daughter chromosomes have four arms since the fiber attachment is more or less median, while the other four daughter chromosomes consist of the two sister chromatids attached at one end by the fiber constriction (Fig. 9). The nucleolus disappears at metaphase.

The constant association of the nucleolus with one of the shorter chromosomes enables this particular chromosome to be recognized at all stages up to metaphase. This chromosome at late pachytene is shown at the left in figure 10 to show the relative amount of contraction which occurs between pachytene and late diakinesis. The

decrease in length is somewhat more than one-half. Five of the marked chromosomes are shown at the diplotene stage (Fig. 10) for comparison with the same chromosomes at diakinesis (Fig. 11—the five chromosomes to the right). An intermediate stage is also included at the left. It is apparent that there is considerable reduction in the number of nodes, most of which are presumably chiasmata, between diplotene and diakinesis. In the five diplotene chromosomes the average number of nodes is about three per chromosome, while at diakinesis it is only a little more than one per bivalent. An examination of 26 of these marked chromosomes at diplotene showed an average of 2.1 nodes per bivalent while 28 bivalents at diakinesis had an average of 1.1 nodes or chiasmata. For these short chromosomes the reduction of nodes is about 1 per bivalent, or one-half of the nodes formed at diplotene. The long chromosomes may have as many as four or five nodes at diplotene but seldom more than two at diakinesis.

While this evidence is not conclusive it does indicate that there is considerable reduction in the number of chiasmata between early diplotene and the first meiotic metaphase stage.

THE BEHAVIOR OF THE NUCLEOLUS

At the earliest prophase stages of meiosis in *Callisia* only one nucleous is observed. The nucleolus is almost invariably at the periphery of the spireme and attached to the end of a spireme thread. As soon as the spireme is opened up enough to follow the threads it is found that no continuous spireme exists. There are six distinct chromosomes at this stage and the nucleolus is attached to one of these chromosomes, and later stages show that the same chromosome is always involved. As the chromosomes contract the nucleolus disintegrates and it finally disappears at metaphase.

It is apparent that the nucleolar contents cannot pass into the entire spireme thread. It is doubtful if the spireme thread is ever continuous in either the somatic or meiotic prophases of *Callisia*. The fact that the nucleolus disappears or decreases in size and staining capacity as the chromosomes develop has led to the belief that the nucleolar material is absorbed by the chromosomes. A review of the work on nucleolar activity has recently been presented by Zirkle (1931) and by Fikry (1930).

Fikry has presented what appears to be a logical explanation of the relation between nucleoli and chromosomes, and this interpretation seems to have considerable cytological support. The gene string builds up a surrounding sheath of chromatin as the chromosomes develop from early prophase. Each gene builds up its own

specific enzyme complex. At telophase this chromatic product is released from the gene strings, or chromatids, to form the nucleolus. In the next division the nucleolar substance passes out into the cytoplasm carrying with it the specific gene enzymes or enzyme products.

Certainly this theory offers an attractive hypothesis to account for the transmission of gene activity to the cytoplasm. The genes develop a chromatin sheath which is later differentiated into the more chromatic chromonemata and the more or less diffuse chromatin or oxychromatin. As the chromosomes pass to the poles at telophase the chromosome sheaths or pellicles fuse to form the nuclear membrane and the more fluid chromatin matrix is released to form nucleoli (Bridges in Alexander 1928). In cases where the chromosomes are not closely associated at telophase, as in the gymnosperms, many nucleoli are formed, but in most angiosperms the chromosomes are compact at telophase and the released oxychromatin fuses to form one or two nucleoli. The nucleoli become still further transformed so that they no longer take the typical chromatin stains (Zirkle 1931). As the chromosomes develop for the next division the nucleolus usually disintegrates and when the nuclear wall breaks down the products of the nucleolus become incorporated in the cytoplasm. Occasionally the nucleolus persists until metaphase where it may pass to one pole, or divide and pass to both poles, but in either case it usually passes into the cytoplasm and is absorbed. (Yamaha and Sinoto 1925.)

THE MECHANISM OF CROSSING OVER

A modification of Janssen's (1924) "partial chiasmatypy" theory of crossing over has recently been sponsored by Darlington (1930), Maeda (1930) and Belling (1931, a. b.). This theory postulates that a chiasma is caused by crossing over between two of the four chro-

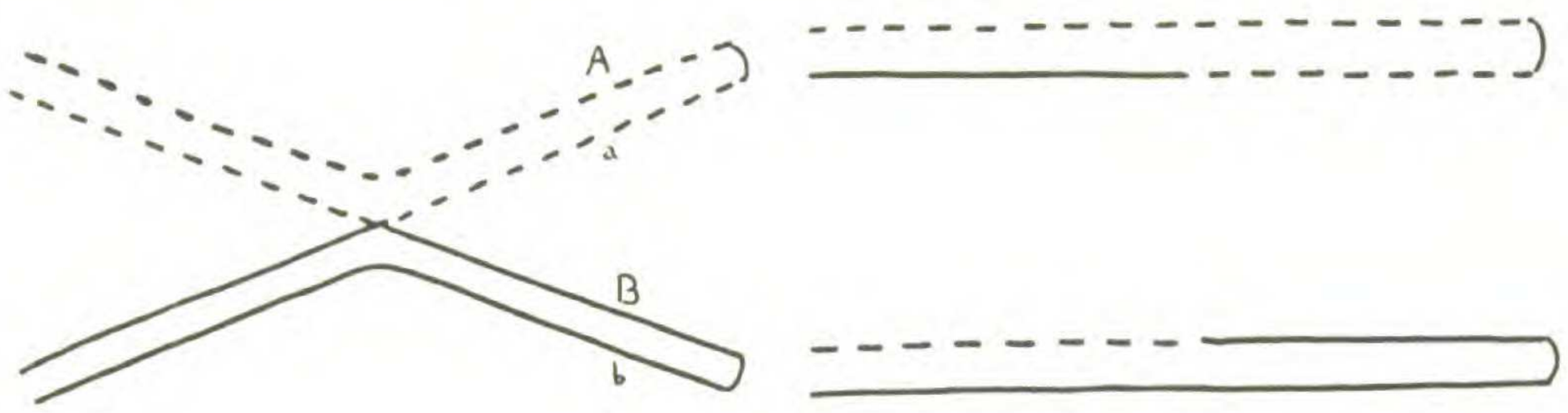


DIAGRAM 1

matids at pachytene. At diplotene the chiasmata represent crossovers and only sister chromatids are paired as shown in diagram 1. When the homologues are separated at metaphase the crossover at

the chiasma will result in two non-crossover chromatids and two crossover chromatids. Every chiasma represents a crossover, but according to Maeda and Belling every point of contact or node at diplotene is not necessarily a chiasma, but may be an overlap or temporary fusion of the paired chromatids. The assumptions upon which this theory is based have been criticized by McClung (1927). The more recent arguments of Belling and Darlington will be considered later in this paper.

In 1930 the writer presented an hypothesis to account for the mechanism of crossing over which seems to have considerable cytological support and is in accord with the genetic requirements. This theory is based on the usual assumption that chiasmata are formed by the alternate opening out of sister and non-sister chromatids at diplotene. A crossover is caused by a break in the two crossed chromatids at a chiasma between diplotene and late diakinesis. (Diagram 2.)

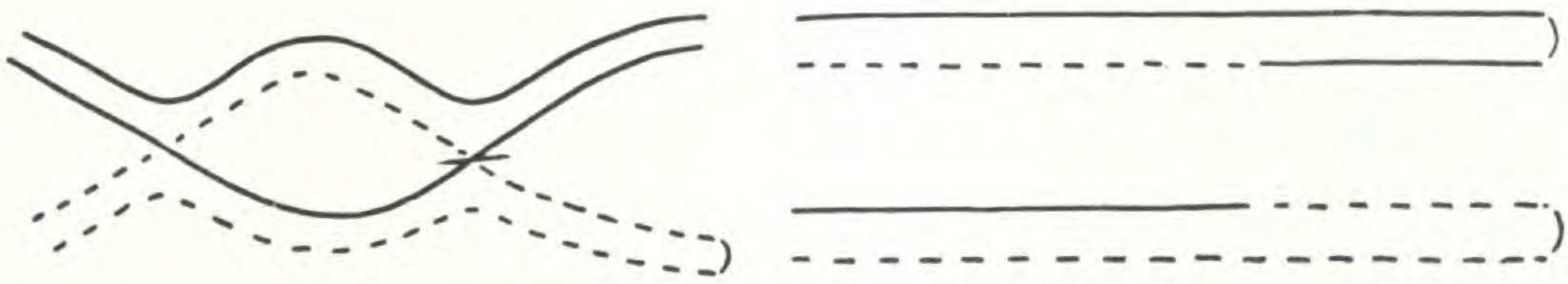


DIAGRAM 2

It is assumed that sister chromatids are always associated at the spindle fiber attachments during the first meiotic civision, which is in accord with both the cytological and genetic evidence. The second meiotic division separates the sister chromatids and is an equational division for regions of the chromosome between the spindle fiber and the first crossover.

The paired chromatids are assumed to be associated, gene by gene, throughout their length, so that a crossover usually occurs between the same two consecutive genes in each chromatid as shown in diagram 3. The spindle fiber is to the right and is terminal.

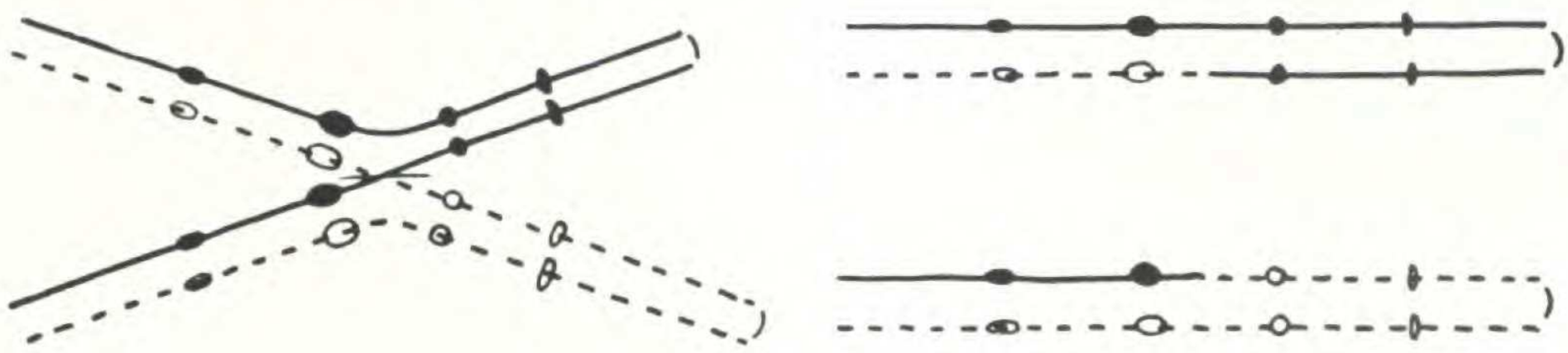


DIAGRAM 3

In rare cases unequal crossing over occurs (Sturtevant 1925, 1928, Morgan 1931) which can be accounted for if the genes in the region

of the chiasma are not closely paired. In such a case crossing over need not occur between the same two consecutive genes in each chromatid, but may result in gene duplication and deficiency as shown in diagram 4.

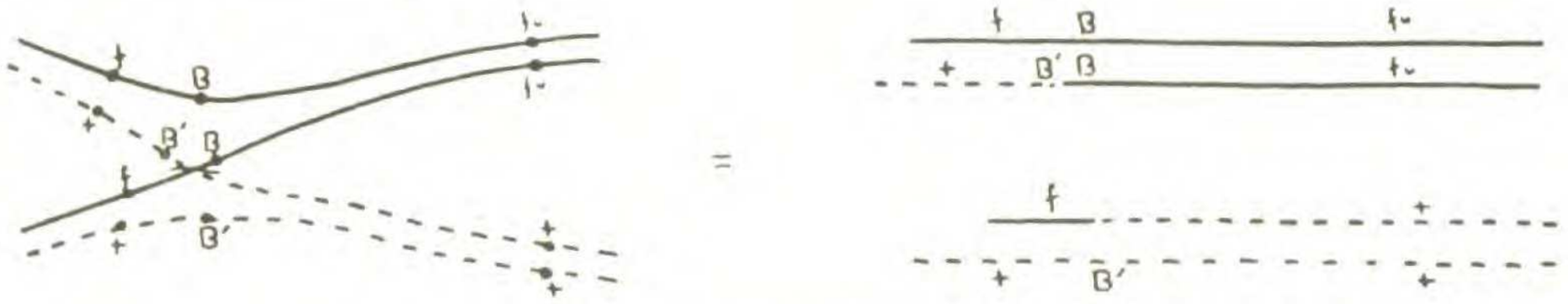


DIAGRAM 4

Gene duplication and deficiency occur in case of the bar gene in the X chromosome of *Drosophila* only when there is crossing over between forked and fused, so Sturtevant concluded that crossing over does not occur between sister chromatids in this region of the X chromosome. The order of the two duplicated genes bar and infrabar may be BB′ or B′B. In diagram 4 the order of these genes is BB′ but if the chromatids cross and break above these two genes the order will be B′B. All of these genetic observations can readily be explained on the writer's hypothesis.

Crossing over between sister chromatids, according to this theory, cannot occur before the first regular genetically detectable crossover, and at other points can occur only when a half twist occurs in one pair of sister chromatids accompanied by an opening out of non-sister pairs of chromatids on both sides of the half twist. (Diagram 5.)

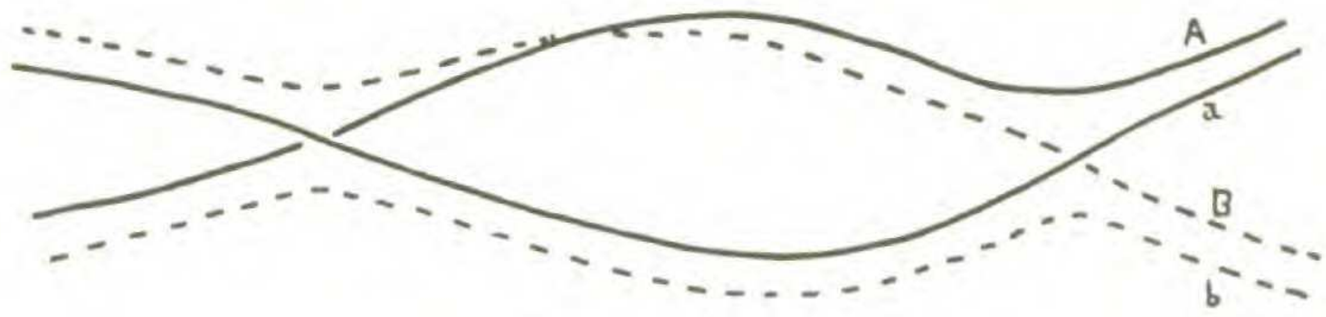


DIAGRAM 5

The first crossover from the spindle fiber end of the chromosome would invariably occur between non-sister chromatids, but in some cases subsequent crossovers might occur between sister threads. Thus there would be little chance for a sister thread crossover to occur at the bar locus in the X chromosome of *Drosophila* because bar is only about 12 units from the fiber attachment point. The hypothesis is again in accord with the genetic observation that crossing over does not occur between sister chromatids at the bar locus (Sturtevant 1928).

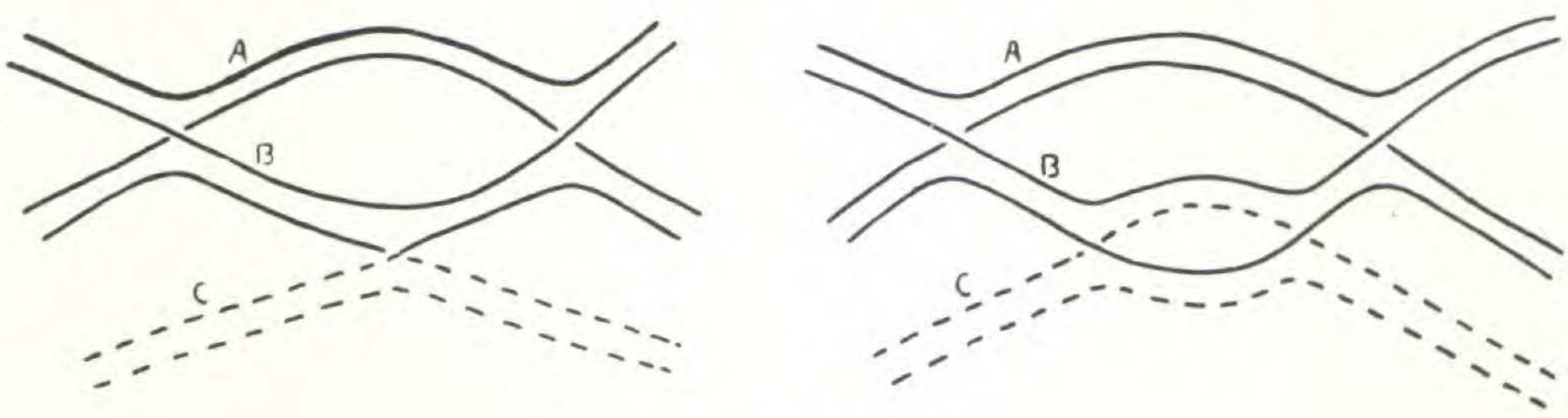
Factors which suppress crossing over in *Drosophila* (Gowen 1922, 1928) also inhibit chromosome pairing. Crossover reducers are known to be caused by inverted segments (Sturtevant 1926) trans-

locations (Dobzhansky 1931) and may be caused by deficiencies. Any factor which inhibits chromosome pairing would of course decrease or eliminate crossing over between homologous chromosomes on either theory of crossing over.

JANSSEN'S "PARTIAL CHIASMATYPY" HYPOTHESIS

According to Darlington (1930) "partial chiasmatypy," which demands crossing over between two of the four chromatids at every chiasma, is the only possible working hypothesis for the correlation of the cytological and genetical observations." Darlington's argument is based on the types of chromosome association in triploids and tetraploids. The drawings and diagrams in his 1930 paper "are the result of a special search for the missing configuration in this critical material." In *Tulipa* and *Hyacinthus* triploid chromosomes were occasionally found to be associated as shown in diagram 6. A third chromosome intercalated between two chiasmata of a bivalent by a single chiasma is assumed to be critical evidence that this chiasma is caused by a previous crossover between two chromatids. If a third chromosome in triploids is invariably intercalated between two chiasmata in the other two chromosomes by a single chiasma there might be some justification for Darlington's conclusion, but since the frequency of such types is not known, the evidence presented is not critical.

These types of trivalents can also be explained on the assumption that chiasmata are caused by alternate opening out of sister and non-sister chromatids and that one chiasma disappears before diakinesis due to breaks in the chromatids as shown in diagram 7.



DIAGRAMS 6 AND 7

According to this theory of chiasma formation chromosome C must form two chiasmata with chromosome B. But if one of these chiasmata frequently breaks, as would be expected according to the writer's theory of crossing over, then at diakinesis chromosome C will often be associated with B only by a single chiasma. Darlington's tetraploid configurations and *Oenothera* figure 8 chromosomes (Darlington 1931 B) can be interpreted in the same way, and cannot

be considered as critical evidence in favor of Janssen's partial chiasmatypy theory of crossing over.

If all three homologous chromosomes in triploids can pair simultaneously at the same locus, as Belling has described in *Hyacinthus*, then Darlington's "critical" figures could be obtained without any crossing over.

In a later paper Darlington (1931a) attempts to correlate the frequency of chiasma formation with the percentage of crossing over in *Primula sinensis*. The SBGL chromosome, which is assumed to be one of the longest chromosomes of *Primula sinensis*, was estimated from crossover data, to be 111.6 genetic units long, but this may be incorrect since Haldane's (1919) correction of map distance is not valid (Morgan, Bridges and Sturtevant 1925).

According to Darlington's theory of crossing over each chiasma will result in 50 per cent crossing over and the length of a chromosome in genetic units can be calculated as 50 × the number of chiasmata. Darlington finds an average of 3.5 chiasmata per bivalent which he considers may be an under-estimate for the longest chromosomes. Due to an error, Darlington calculated the crossover length, based on number of chiasmata, as 58.3 to 116.7 units, which he says is "in strict agreement with expectation." But the correct length based on chiasma frequency, should be 3.5 × 50 or 175 units as an average, or to range from 116.7 to 233.4 units, as Darlington later discovered. Obviously the length of the longest chromosome as measured by crossing over is only about half as long as the length expected, if each chiasma represents a crossover.

On the writer's theory of crossing over, chromosome SBGL should be somewhat more than 100 units long which is "in strict agreement with expectation"! It is probable however, that neither the genetic nor the cytological data are adequate for any serious calculation of the crossover length of any of the chromosomes in *Primula*.

In order to explain the absence of crossing over in the *Drosophila* male, Darlington (1931 a) assumes that there are always two chiasmata in each bivalent, that the two chiasmata are very close together, that no mutations occur in the region between chiasmata, that the spindle fiber attachment point is between the chiasmata, and that the double crossover invariably involves the same two chromatids. All of these assumptions are highly improbable, and the assumption that the two crossovers are always reciprocal is not in accord with the genetic evidence.

On the alternative theory of crossing over, it is assumed that in the *Drosophila* male, chiasmata are formed as they are in the female, although perhaps less frequently, and that chromosome develop-

ment from diplotene to metaphase is sufficiently gradual so that the chromatids can adapt themselves to the changes without breaking. A gradual development would permit the chromatids to contract as the chromosomes contract so that no coiling of the chromonemata would occur. The chiasmata would be free to terminalize without breaks in the chromatids, as seems to be the case in the Orthopteran chromosomes. Since terminalization is easily effected the meiotic metaphase in the male should be of short duration as compared with the same stage in the female, as seems to be the case (Huettner 1930). The reverse would be the case according to Darlington's theory. Crossing over in the female is most frequent towards the ends of the second and third chromosomes of *Drosophila melanogaster* so that only two subterminal chiasmata would usually need to be pulled apart, but in the male the two reciprocal chiasmata near the spindle fiber would have to be terminalized for practically the entire length of the chromosomes.

Darlington does not attempt to explain why breaks occur in the chromatids at pachytene, why the breaks in the crossover chromatids almost invariably occur at the same level, why chromatids should recombine in new association after they break, why only two chromatids cross over at any one locus, or why one crossover interferes with the occurrence of a second one in the same region.

According to Darlington, at least one chiasma is essential for pairing of homologous chromosomes and every chiasma represents a crossover. Therefore, crossing over must be universal in all normal species and no normal chromosome can be less than 50 genetic units long. No explanation has been presented to account for the behavior of the fourth chromosome of *Drosophila melanogaster*. This chromosome is about as regular in conjugation and disjunction as the X chromosome, but no crossing over occurs in the fourth chromosome. There are also other obvious objections to Darlington's theory of chromosome pairing (O'Mara, in press).

BELLING'S THEORY OF CROSSING OVER

Belling (1931 a) has recently presented an hypothesis to account for the mechanism of crossing over. The homologous chromosomes pair as single chromatids. Half twists occur in the paired homologues at early pachytene before the secondary split has begun. When the secondary split occurs the new chromomeres must form new connecting fibers and at each twist they take the shortest route in connecting adjacent chromomeres. Thus crossing over would occur only between the two new chromatids formed at late pachytene. This interpretation is obviously invalid because in *Drosophila*

crossing over is found in more than 50 per cent of the emerging X chromosomes, and nearly 75 per cent of the third chromosomes have one or more crossovers (Redfield 1930).

More recently Belling's (1931 b) modified his hypothesis. This new theory seems to be plausible since it accounts for crossing over between any two chromatids, and provides a mechanism to explain translocations and inversions in somatic chromosomes. If Janssen's partial chiasmatypy theory of crossing over is correct Belling's hypothesis of the mechanism involved would seem to be the only logical explanation. Unfortunately there are a number of serious objections to this theory.

According to Belling a half twist between single chromatids occurs at pachytene. When the new chromomeres are produced at the secondary split the connecting fiber between genes may remain with the old gene (genes and chromomeres are assumed to be synonymous) according to the laws of chance. The new connecting fibers then unite the free genes by the shortest path (Diagram 8).

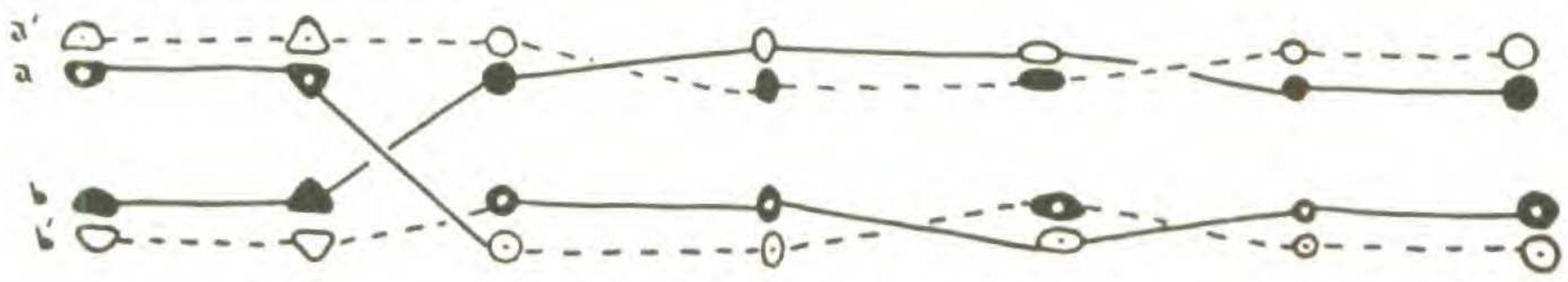


DIAGRAM 8

As shown in the diagram 8, the new connecting fibers may result in a crossover between a′ and b′ or between a′ and b. If the connecting fibers remain with the old genes or pass to the new ones at random there will be random crossing over between any two of the four chromatids.

Random assortment of connecting fibers would also mean that in both somatic and meiotic chromosomes crossing over between sister threads would be very frequent. In a chromosome containing a hundred genes 50 sister crossovers would be expected. In the somatic chromosomes this sister crossing over would result in much twisting of the two chromatids at late prophase and at metaphase, if the new connecting fibers which unite old and new genes pass at random on either side of the old connecting fiber. But there are only a limited number of twists or half twists in the somatic chromosomes (text figure 1) and between paired chromatids at meiosis (Seiler, 1926).

Belling's theory is also based on the assumption that the homologous chromosomes first pair as single chromatids at meiosis. The work of Kaufmann (1926) and Sharp (1929) seems to show that the

somatic chromosomes in certain plant species are already split into two chromatids at the preceding anaphase. According to Kaufmann (1931) the chromosomes at the telophase of the last premeiotic division show the parallel chromonemata. In *Paratettix*, the chromosomes are split longitudinally when they enter the spermatid (Robertson, 1931 a), and Robertson (1931 b), also shows that the homologous chromosomes in the *Tettigidae* are already divided into sister chromatids when they begin pairing at meiosis. Similar observations have previously been made by Robertson (1916) and McClung (1928). These observations cannot be reconciled either with Belling's theory of crossing over, or with Darlington's (1931) theory of meiosis.

One other point in comparing the two theories has been presented by Belling (1931 b) in connection with the behavior of unequal homologues. One pair of unequal homologues were found in *Aloe purpurascens*. If chiasmata are formed by the alternate opening out of sister and non-sister pairs of chromatids, in some cases a short arm should be associated with a long one at diplotene or diakinesis, but no such association was found. On Belling's hypothesis no pairing of long and short arms would be expected since only sister chromatids are paired at diplotene. If the chiasma is to the distal side of the fiber constriction, as shown in diagram 9b, then a short and a long arm should be associated on the writer's hypothesis, unless a crossover had occurred. On Belling's hypothesis such a chiasma (9a) should invariably result in a distribution of a long and a short chromosome to each pole at the first meiotic division, but Belling observes that "in some cases they are observed to separate into short plus long and short plus long chromatids."

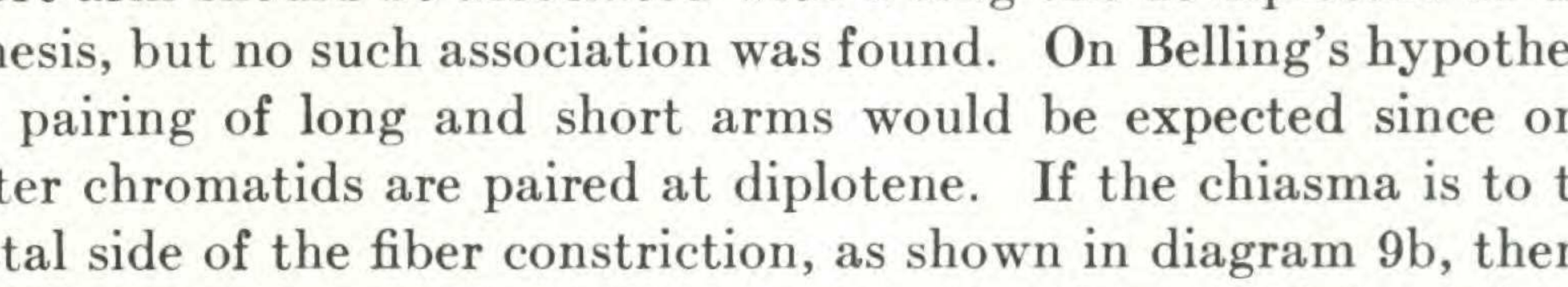


DIAGRAM 9

If, in most cases, the segregation at anaphase is into two short and two long homologues, as Belling's statement would imply, then the chiasma must be to the right of the fiber attachment point (9c) or the homologues are terminally associated without chiasma formation. On the writer's hypothesis a chiasma to the right of the fiber constriction (9b) or a terminal association of chromosomes, would result in the observed association of chromatids—short with short and long with long at diplotene. It is evident that Belling's obser-

vations are inadequate for any critical test of the method of chiasma formation.

REDUCTION IN NUMBER OF CHIASMATA

The writer's theory of crossing over postulates breaks in the chiasmata so that a decrease in the number of chiasmata would be expected between early diplotene and metaphase if crossing over occurs.

At early diplotene, as the homologous chromonemata open out, the nodes and internodes are often very numerous. This condition is indicated in chromosome number 3 in figure 2, and is clearly shown in *Lathyrus* (Maeda, 1930, Fig. 17 B) and in *Zea* (Cooper and Brink 1931, Fig. 1). These nodes may be chiasmata, most of which subsequently meet as the homologous chromonemata open out more completely at the internodes. There is also a possibility that some of these nodes which disappear are due to breaks in chiasmata at this stage of meiosis. Since the individual chromatids cannot be identified many of these early nodes may be only temporary adhesions as Belling has suggested.

In *Tulipa* Newton (1926) pictures about 30 chiasmata in 11 diplotene chromosomes (Fig. 25). At early diakinesis the number of chiasmata are especially clear, and show a total of about 27 chiasmata for the 12 chromosomes (Fig. 31) while at late diakinesis Newton found only 15 chiasmata (Fig. 30). Thus the average number of nodes or chiasmata per bivalent chromosome is reduced from almost 3 at diplotene to only 1.25 at late diakinesis.

In *Lilium longiflorum*, Belling (1928) found a decrease in number of nodes between diplotene and late diakinesis of 43 per cent and suggests that more nodes would have been found if counts could have have been made at an earlier stage. Belling states that "the nodes which disappear between diplotene and late diakinesis do not seem to be all or mainly twists."

In a later paper Belling (1931) concludes that in the same species of *Lilium* the number of nodes is reduced from 42.5 at diplotene to about 30 at late diakinesis and metaphase, and suggests that the nodes which disappear are half-twists or temporary adhesions rather than chiasmata.

Darlington (1931 a) finds an average of 3.5 chiasmata at diplotene in *Primula sinensis* but only 1.89 chiasmata at metaphase. The bivalents at metaphase are associated only by terminal chiasmata. This decrease in number of chiasmata is attributed to terminalization.

Secale chromosomes at diplotene may often have four or five nodes, but never more than two at diakinesis and metaphase (Sax 1930).

In *Rosa blanda*, Erlanson (1931) obtains an average of 1.94 chiasmata per bivalent at early diakinesis, but only 1.31 at metaphase. Doubtless a greater number of chiasmata would have been found at diplotene.

In *Matthiola* Philp and Huskins (1931) found that the average chiasma frequency was 2.26 per chromosome at early diakinesis and only 1.54 at metaphase.

The counts of chiasma frequency at different stages of meiosis are summarized in Table 1. The estimated average crossover length of the chromosomes is calculated from the number of chiasmata which disappear between diplotene and metaphase on the basis of the writer's hypothesis. Since Belling considers that the chiasmata found at metaphase constitute all or most of the true chiasmata formed, the crossover length is also calculated on this basis. Darlington considers the nodes found at diplotene in *Primula* as chiasmata and attributes the loss of chiasmata to terminalization, so crossover lengths are also calculated on diplotene and early diakinesis counts.

TABLE 1

	Ave. no. xta. per bivalent			Estimated Ave. c.o. length		
Genus	Diplotene or early diakinesis	Metaphase	No. lost	S.	B.	D.
Tulipa.....	2.8	1.3	1.5	75	65	140
Lilium.....	3.5	2.5	1.0	50	125	175
Secale.....	3.5	1.8	1.7	85	90	175
Primula.....	3.5	1.9	1.6	80	95	175
Rosa.....	1.9	1.3	.6	30	65	95
Matthiola.....	2.3	1.5	.8	40	75	115
Callisia.....	4.2	1.5	2.7	135	75	210

Counts of chiasma frequency at early diakinesis do not represent the number formed at early diplotene so that in most of the genera listed above, the number of chiasmata lost should be increased considerably and consequently the crossover length would be increased on the writer's and on Darlington's hypotheses. Little is known concerning the crossover lengths of the chromosomes of the species listed above, but the calculated lengths are comparable to those found in *Drosophila* and *Zea*. Two of the autosomes in *Drosophila melanogaster* are somewhat more than 100 units long and in *Zea* the 5 chromosomes containing the most mutations range from about 50 to 68 units long (Lindstrom 1931). The chiasma frequency in *Zea* chromosomes is about 1.5 at metaphase (Randolph), but data on earlier stages are very meagre although Fisk (1927, Fig. 32) pictures two bivalents with about 5 nodes each. The genetic and cytological work on *Zea* should soon provide adequate data for a critical comparison of chromosome behavior and crossover frequency.

Darlington assumes that the reduction in number of chiasmata is due to terminalization. But as Belling (1931 b) points out, the coiling of the chromonemata would prevent any appreciable movement of the chiasmata after diakinesis. No coiled chromonemata have been described by Darlington, probably due to inadequate fixation or staining for showing this structure, rather than the absence of coiled chromonemata in the species studied. Coiled chromonemata have been described in many species and recently Shinke (1930) has described such chromosome structure in about 25 different genera. The coiling of the chromonemata probably begins at late diplotene so that no extensive movement of the chiasmata can occur, in most cases, between diplotene and metaphase. If it is assumed that the chiasmata are terminalized one might expect that all of them would frequently terminalize before metaphase and produce univalent chromosomes. In *Primula*, and in the *Solanaceae*, the bivalents at metaphase are usually associated only at the two ends forming the typical ring shaped bivalent. Darlington assumes that an average of about 1.5 chiasmata pass off the ends of the *Primula* bivalents. But why should the remaining two chiasmata always stop at the ends of the bivalent?

If each chiasma represents a crossover then the M chromosome of *Vicia faba* must be more than 400 crossover units long. Maeda (1930) finds an average of 8.1 chiasmata in this chromosome, and the number may be as high as 13 in some cases. On any theory of crossing over a twist must occur in the chromatids either at the chiasma or at an internode for every crossover. If one or more crossovers occur there should often be some interlocking of homologues at anaphase as seems to be the case in *Lilium* and *Lathyrus* (Maeda 1930, Sax 1930). With 8 crossovers one might expect considerable difficulty in separating homologues at the first meiotic division. If the chiasmata are not the result of crossovers there would of course be no difficulty in the division of homologues. (McClung 1927).

NON-DISJUNCTION AND CROSSING OVER.

Non-disjunction in *Drosophila melanogaster* was first described by Bridges in 1916. Primary non-disjunction is caused by the production of "2-X" and "no-X" eggs which when combined with normal sperm produce male and female exceptions. These primary exceptions occur with a frequency of about 1 in 2000. Male exceptions are usually produced from 4 to 8 times as frequently as female exceptions (Bridges 1916, Safir 1920, Mavor 1924, Anderson 1931). The excess of male exceptions, which are produced from

"no-X" eggs, is attributed to the failure of the XXs to pass to either pole at the first reduction division.

In normal stocks of *Drosophila* little if any crossing over takes place between the two X chromosomes which pass to the same egg. (Bridges 1916.) In high non-disjunction lines, crossing over is greatly reduced in both the normal and in the exceptional progeny (Morgan et al. 1925). In Anderson's (1929) high non-disjunction stock crossing over between scute and forked was reduced from 62 per cent to 20.9 per cent, and in the XXs which produced exceptional females the crossing over was only 7.3 per cent. Crossing over was almost eliminated in the region of vermillion, 40 units from the left end of the X, but increased towards the ends and was almost normal at the left end. Dr. Anderson informs me that the reduction in crossing over was caused by a translocation involving the X and the third chromosome. Dobzhansky (1932) also finds that translocations reduce crossing over and that non-disjunction is positively correlated with the length of the autosome attached to the Y. Due to the attraction of different chromosome segments chromosome pairing is often weak and crossing over is reduced.

In Anderson's (1931) primary exceptions produced by X-rays the total crossing over between scute and forked was about 60 per cent of the normal, but crossing over was found in all regions, and in two of the regions studied crossing over was almost normal. About 14 per cent of the XXs were homozygous for the forked locus where less than 5 per cent would be expected. This excess of homozygosis at forked is attributed to non-disjunction at the second maturation division, the only logical explanation of the results obtained. In view of the great irregularity of crossing over in different regions compared with the control it seems possible that there was 6 per cent of crossing over to the right of forked. At any rate crossing over between the XXs which pass to the same egg, is not confined to the distal ends of the chromosomes and some crossing over occurs to the right of forked.

In non-disjunction is due to a failure of XXs to separate at the first maturation division it is rather difficult to account for the decrease in crossing over in high non-disjunction stock if chiasmata represent crossovers. According to Darlington (1931) chiasmata form the only bonds between homologous chromosomes and if no chiasmata are formed the chromosomes would not be expected to form bivalents at meiosis. Failure of the XXs to disjoin would then be attributed to more than the usual number of chiasmata, but an excess of chiasmata would also mean an excess of crossing over on either Belling's or Darlington's interpretation. On the writer's

interpretation the decreased amount of crossing over in high non-disjunction lines could be attributed to few breaks at chiasmata so that the homologues would be united by more than the usual number of chiasmata and could not separate so readily.

There is good evidence, however, that non-disjunction is really a failure of chromosome pairing at the later stages of the first meiotic divisions. The decreased crossing over in high non-disjunction strains of *Drosophila*, and in the XXs which pass to the same egg in normal stock, can be attributed to non-conjugation or to pairing in only restricted regions of the chromosome. If only a few chiasmata are formed, due to incomplete pairing, then crossing over would be reduced on either theory of crossing over. The premature separation of homologues could be attributed to either early terminalization of these few chiasmata, or to breaks which would result in cross-overs. On Darlington's and Belling's theory, all of the chiasmata must be prematurely terminalized. The failure of chiasma formation, or breaks the few chismata formed, would produce unpaired homologues at the first maturation division. If these univalents pass at random to either pole, then half of the eggs should contain no X or 2 X chromosomes, and half should contain the usual 1 X. The univalents often fail to reach the poles in species hybrids and in haploids, and are often lost in the cytoplasm. If then one of the two X chromosomes should be lost there will be an excess of no-X eggs which produce the male exceptions. Such a behavior of the univalents would account for the excess of male as compared with female exceptions. This explanation of non-disjunction seems to be more plausible than the assumption that the two XXs are so intimately paired that they fail to divide and both pass to the same pole or fail to reach either pole.

If non-disjunction is due to a failure of the X chromosomes to separate it is difficult to account for the decrease in crossing over in high non-disjunction lines of *Drosophila* on the theories of crossing over proposed by Belling and by Darlington. On the other hand if non-disjunction is due to non-conjugation at metaphase, as seems probable, then all chiasmata must be broken or prematurely terminalized. If chiasmata are crossovers then all of the XX chromosomes which show crossing over must have separated prematurely by complete terminalization and elimination of all chiasmata. In the high non-disjunction stock of Anderson's about 7 per cent of the crossovers between the two XXs which pass to the same egg occur to the right of forked, and in the X-ray material 6 per cent of the crossovers may have occurred to the right of forked. In most of these forked equationals an additional crossover also occurs

to the left of forked. If crossovers produce chiasmata then in these chromosomes there are usually two chiasmata which must be terminalized to produce "non-disjunction" and one of the chiasmata is to the right of forked so that it would have to be prematurely terminalized for practically the entire crossover length of the chromosome. If such terminalization is possible it would seem that non-disjunction should be frequent, but primary exceptions occur with a frequency of about one in 2000, in normal stocks of *Drosophila*, and in only 2.5 per cent of the progeny from high non-disjunction lines. The cytological evidence also indicates that extensive terminalization or movement of chiasmata is improbable (Belling 1931 b).

If Painter's (1931) cytological map of the X chromosome is correct there is some possibility that premature terminalization could occur because the region from scute to forked constitutes only about a third of the cytological length of the X chromosome. Stern (1931) working with the same stock finds that the region from scute to forked constitutes about one-half of the X chromosome and his figures clearly support this interpretation. It would seem improbable that two chiasmata could be prematurely terminalized, one for more than half the length of the X chromosome. Even in species where the homologous chromosomes are always associated by only terminal chiasmata at metaphase there is little or no tendency for premature separation of the chromosomes.

On the writer's theory of crossing over it is not difficult to explain "non-disjunction" even where crossing over occurs near the spindle fiber end of the X chromosome. If few chiasmata are formed at diplotene and all of them break in certain bivalents, then these chromosomes will be loosely associated so that precocious disjunction before metaphase would be possible.

There are, however, certain types of double crossovers that are difficult to explain unless some terminalization of chiasmata occurs. In Anderson's high non-disjunction data there is 1 and in the X-ray data there are 4 double crossovers of the type $\frac{aab}{aba}$ or $\frac{bba}{bab}$. These reciprocal-equational double crossovers can only occur, on the writer's hypothesis, if there is an unbroken chiasma between sister chromatids between the first and second genetically detectable crossovers, or a twist in the sister chromatids between the second and third crossovers. In Anderson's (1931) table 3, one of these reciprocal-equational crossovers is the result of a second and third crossover since the forked locus is homozygous, but the other three double crossovers apparently involve a first and second crossover

because forked is heterozygous in these cases. Under such conditions it would be necessary to assume that a chiasma between sister chromatids must be prematurely terminalized to produce "non-disjunction." Since these chiasmata would be to the left of forked and in two cases to the left of cut, it is not impossible that premature terminalization might occur. It is also possible that these three reciprocal-equational crossovers are produced by second and third crossovers, and that the first crossover was a reciprocal to the right of forked, or that these exceptional crossovers are produced by the non-disjunction at the second maturation division.

The writer's hypothesis offers a simpler and more plausible explanation of "non-disjunction" than Janssen's partial chiasmatypy hypothesis, regardless of which interpretation of "non-disjunction" is correct.

RANDOM CROSSING OVER BETWEEN THE FOUR CHROMATIDS.

Crossing over is not limited to two of the four chromatids, because more than 50 per cent of the X and third chromosomes of *Drosophila* which emerge from the reduction division, have one or more crossovers. In 62 units of the X chromosome 54 per cent of the emerging chromosomes show at least one crossover (Anderson and Rhoades, 1931), and in the third chromosome 72.9 per cent of the chromosomes are crossovers (Redfield, 1930). If crossing over occurs only between the new chromatids as Belling (1931 a) has suggested, then not more than 50 per cent of the emerging chromosomes should be cross overs, regardless of the map length of the chromosome.

More recently Belling (1931 b) has assumed that "the old connecting fiber is indifferent as to which chromiole it will remain with." On this assumption crossing over would be at random between the four chromatids.

Belling's hypothesis can be modified to comply with certain cytological and genetic data. If the old connecting fibers usually remain with the old genes at the time the new chromatids are formed, then crossing over will usually occur between the two new chromatids as Belling (1931 a) has assumed. In some cases the old connecting fiber will unite a new and an old gene so that crossovers will occur between sister chromatids. Such crossovers, if sufficiently numerous, would result in random crossing over even if all crossovers at chiasmata were between the two new chromatids. Crossing over between sister chromatids would not be dependent on chromosome pairing and would be expected to occur with equal frequency in all regions of the chromosome.

On the writer's hypothesis random crossing over among the four chromatids can occur only if half-twists are sufficiently numerous in the paired sister chromatids. If the chromosomes are already split into sister chromatids at the time of meiotic pairing it would seem improbable that the same two non-sister chromatids would be paired throughout their length. If the secondary split occurs after pairing it would also be improbable that the sister chromatids would lie parallel throughout their length. One would expect some twists in the paired sister chromatids in either case. Such twists are found in both somatic and meiotic chromosomes.

In normal stocks of *Drosophila* and in cases of "non-disjunction," the genetic assortment of chromatids will be at random for the first crossover, because of their free assortment at the second maturation division. In attached-X stock, however, the random distribution at both first and second crossovers can be tested.

Random crossing over can occur in attached XXs, on the writer's theory, only if twists in paired sister chromatids are sufficiently numerous. Such half-twists could occur between the point of spindle fiber attachment and the first chiasma, or between the second and third chiasmata. (Diagram 10.) They could not occur between the first and second chiasmata without the formation of an additional chiasma between sister chromatids, but such chiasmata would be expected only in rare cases.

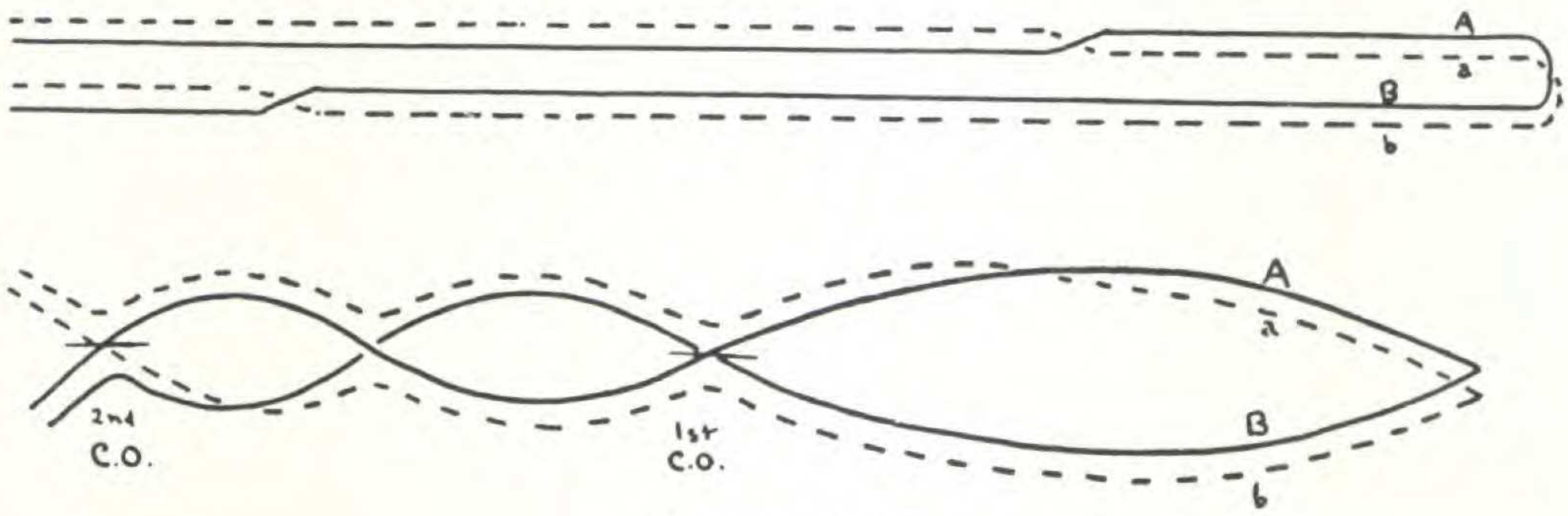


DIAGRAM 10

Only two types of first crossovers need be considered; (0) no twists in either pair of sister chromatids between the attachment point and the first crossover chiasma, and (1 a) one half twist in only one pair of sister chromatids, Aa. The other two types, (1 b) a half twist in chromatids Bb, or (1a–1b) in both pairs of sister chromatids, need not be considered since they are reciprocal to the first two types.

Between the first and second crossovers four types of chromatid association must be considered if crossing over is at random. There may be (0) no twists in either pair of sister chromatids, (1 a) one half-twist in chromatids Aa, (1 b) one half twist in chromatids Bb,

and (1a–1b) a half twist in each of the paired sister chromatids. If such half-twists are frequent there might be several of them between the point of attachment and the first crossover, or between crossovers, but if these twists occur at random they will produce the same types and proportions of crossovers as expected on the assumption that the above four types occur in equal proportions.

If no half twists occur as we would expect types (O) and (1a–1b) to be formed in equal proportions, but no types 1a or 1b would be expected. At a given chiasma crossing over could occur between A and b chromatids, or between B and a. If the first crossover is between A and b the second crossover may occur between A and b or between a and B. With no half twists in pairs of sister chromatids only equational crossovers could occur in attached XXs.

If chiasmata are produced by previous crossovers, the modification of Belling's hypothesis would seem to be the most plausible explanation of the mechanism involved. If sufficient crossovers occur between sister chromatids, the detectable crossovers will be at random between the four chromatids. The association of chromatids shown in diagram 11 will give the same random crossing over as direct crossing over between A and B or b, and B with either A or a.

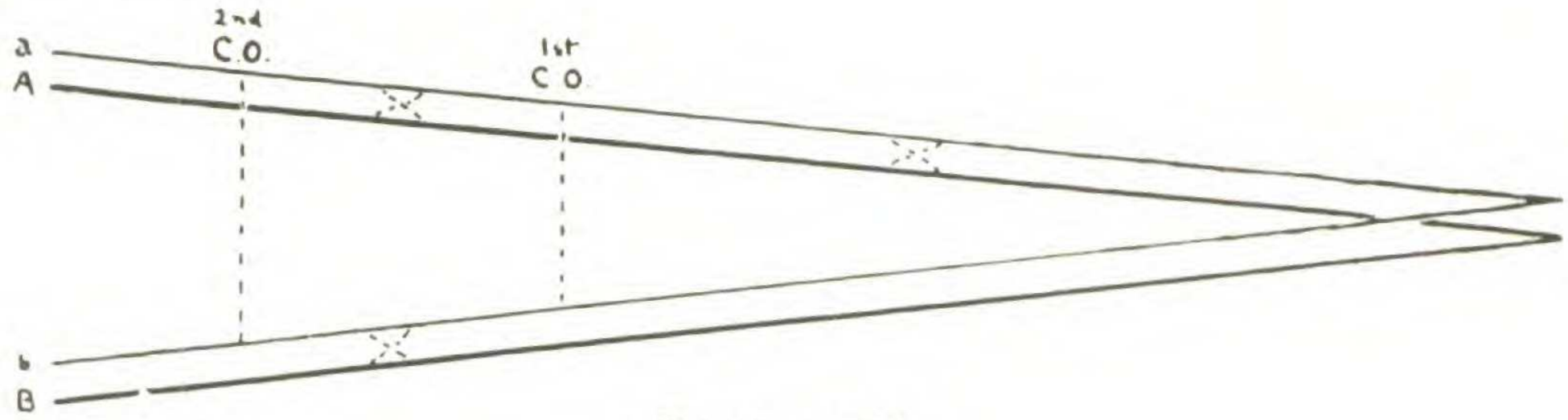


DIAGRAM 11

The detectable crossovers are shown by the lines connecting the two daughter chromatids. Only two types of chromatid association need be considered between the point of attachment of the X chromosomes and the first crossover;—(O) no crossovers occur in either pair of sister chromatids, and (1a) a sister crossover occurs in the Aa pair of chromatids. Between the first and second chiasmata there may be, (O) no sister crossovers, (1a) an excess of 1 sister crossover in chromatid Aa, (1b) an excess of 1 sister crossover in chromatid Bb, or (1a–1b) an equal number of sister crossovers in each of the pairs of sister chromatids.

On either theory we have 8 types of chromatid association in the attached X bivalents, which produce 16 classes of crossovers. The results of random crossing over at two points in the bivalent attached X chromosomes are shown in table 2.

TABLE 2

Types of chromatid association resulting from random crossing over at two points in attached X chromosomes.

C.O. Class	1st C.O. Sax	1st C.O. Bell.	2nd C.O. S. or B.	Crossover types	1st crossovers eq.	1st crossovers rec.	2nd C.O. types 1	2nd C.O. types 2	2nd C.O. types 3	homozygosis 1st Xta	homozygosis 2nd Xta
1	0	1a	0	aaa aba / bab bbb	2			2		2	
2	0	1a	1a	baa aba / aab bbb	2			2		2	
3	0	1a	1b	aaa bba / bab abb	2			2		2	
4	0	1a	1a, 1b	baa bba / aab abb	2			2		2	
5	1a	0	0	aba aaa / bab bbb		1			1		
6	1a	0	1a	aba baa / aab bbb	1	1	1				2
7	1a	0	1b	bba aaa / bab abb	1	1	1				2
8	1a	0	1a, 1b	bba baa / aab abb		2					
					10	— 5	2	— 8	— 1	8/16	4/16

In the second column are listed the types of first crossovers which have been considered on the writer's hypothesis and on the modified hypothesis of Belling. The genetic constitution of the attached XXs which pass to the egg cell are indicated under crossover types. Difference between chromatids A and a, or B and b, are not indicated since they cannot be detected by genetic tests. The first detectable crossovers from the attachment point are shown to occur in the ratio of 2 : 1 if crossing over is at random. Three types of second crossovers occur:—(1) equationals homozygous to the left $\left(\frac{ab}{aa} \text{ or } \frac{bb}{ba}\right)$, (2) equational crossovers homozygous to the right $\left(\frac{ab}{bb} \text{ or } \frac{aa}{ba}\right)$, or (3) reciprocal crossovers $\left(\frac{ab}{ba} \text{ or } \frac{ab}{ba}\right)$. With random crossing over these types should appear in the ratio of 2 : 8 : 1. At the left of the first crossover chiasma one-half of the attached X chromosomes should be homozygous, and one-fourth should be homozygous recessives. At the left of the second crossover chiasma one-fourth of the attached XXs should be homozygous and one-eighth recessives.

If crossing over is at random the first crossovers from the spindle fiber end in attached XX should be equationals and reciprocals in the proportion of 2 : 1 (Table 2). Anderson (1925) found equational and reciprocals in his attached X data, in the proportion of 29.7 to 15.6. Although there is a slight excess of equationals the ration is very near random expectation. If equational and reciprocal first crossovers occur in the ratio of 2.1 then the percentage of homozygous recessives should be half of the crossover distance between the spindle fiber and the first crossover. For forked, which is about 10 units from the spindle fiber, the percentage of recessives in attached XXs was found to be 5.2 by Anderson, 4.9 by Rhoades, and 5.1 by Sturtevant. The genetic evidence indicates that the chromatids are assorted at random at the first crossover.

Random assortment of chromatids would be expected if there is an average of 0.5 or more half-twists or sister crossovers between the attachment point and the first crossover. Even with relatively few half-twists or sister chromatid crossovers in the X chromosome, random crossing over might be expected at the first crossover chiasma because the region between the spindle fiber and the first crossover would usually include more than half of the length of the chromosome (Stern 1931).

The randomness of chromatid association at the second crossover can be determined from the proportions of types of second crossovers in attached X and XXs from "non-disjunction." The non-

disjunction types of crossovers can be derived from table 2 by combining each two crossover combinations into the four possible combinations expected if non-disjunction is due to non-conjugation at metaphase. The classes of crossover combinations will be doubled but the proportion of types of second crossovers will remain the same as shown in table 2.

The available data on types of second crossovers are presented in table 3.

TABLE 3.

Types of 2nd crossovers. (Cf. Table 2)

	(1)	(2)	(3)
Anderson 1925 Table 6	1 (.6)	7 (3.0)	1 (.6)
Anderson 1929 Table 24–25	1 (.05)	6 (.25)	1 (.09)
Anderson 1931 Table 3	3	3	1

The attached X and high non-disjunction data show a lower proportion of type 1 second crossovers than would be expected if crossing over is at random, but the numbers are too small to be of much value. In the X-ray non-disjunction data there is an excess of type 1 second crossovers caused by non-disjunction at the second division.

The third test of randomness of crossing over can be made by comparing the amount of homozygosis in attached X chromosomes with the amount expected on random assortment of chromatids.

The amount of homozygosis at the left end of attached X, or XXs from non-disjunction, is dependent on the number and types of crossovers. The amount of homozygosis at the left of first and second crossover chiasmata is shown in table 2. Fifty per cent of the two emerging X chromosomes should be homozygous to the left of the first crossover and twenty-five per cent at the left of the second.

It is first necessary to calculate the number of chiasmata which produce crossovers. As Belling (1931 b) has pointed out, the relations between crossover chiasmata and crossing over is as follows if crossing over is at random.

TABLE 4

Crossovers in emerging single chromatids

	0	1	2	3	4
Number of crossover chiasmata	0 = 16				
	1 = 8	8			
	2 = 4	8	4		
	3 = 2	6	6	2	
	4 = 1	4	6	4	1

The data from Anderson and Rhoades (1931) table 1 have been used for the calculation of the frequency of chiasmata which produce

crossovers in the bivalent X chromosomes. Crossover recombinations are shown for 26,908 X-chromosomes of which 46 per cent showed no crossing over in the 62 units between forked and scute, 46 per cent were single crossovers, 7.6 per cent were double crossovers, 0.2 per cent were triple crossovers and .01 per cent were quadruple crossovers.

If crossing over is at random chiasma frequency can be obtained from crossover frequency as shown in table 5.

TABLE 5

Calculation of chiasma frequency based on crossover frequency in the X chromosome of *Drosophila*

	Number of crossovers per chromosome				
	0	1	2	3	4
	46%	46%	7.6%	0.2%	.01%
−4th Xta.....	.01	04	.06	.04	x16 = .16
	45.00	45.96	7.54	.16	
−3rd Xta.....	.16	.48	.48	x8 = 1.3	
	45.83	45.48	7.06		
−2d Xta.....	7.06	14.12	x4 = 28.2		
	38.77	31.36			
	31.36				
−1st Xta.....	8.41	x2 = 62.7			

Total chiasmata in bivalents.

None	Single	Double	Triple	Quadruple
8.4%	62.7%	28.2%	1.3%	.2%

Since quadruple chiasmata, if crossing over is at random, should produce crossover chromatids in the proportion of 1 non-crossover, 4 singles, 6 doubles, 4 triples, and 1 quadruple, it is necessary to subtract proportional percentages from each class of crossovers, and repeat for triples, doubles, and singles in proper proportions. Only one chromatid in 16 produced by quadruple chiasmata (which produce crossovers) will be a quadruple crossover, so the percentage of quadruple crossovers must be multiplied by 16 to obtain the number of quadruple chiasmata. Similar calculations are used to obtain chiasmata frequency from single, double, and triple crossovers.

With the above frequency of single, double, and triple chiasmata it is possible to determine the percentage of homozygosis expected 62 units from the spindle fiber end of the two X chromosomes from attached X and non-disjunction stock. The percentage of homozy-

gosis at the left of the first crossover chiasma is 50, the second 25, (Table 2) and the third 37.5, if crossing over is at random. For homozygous recessives these percentages are 25, 12.5, and 18.75 respectively. The following table shows the percentage of homozygous recessives expected 62 units from the spindle fiber attachment in XX chromosomes.

Chiasmata =	Single	Double	Triple
	62.7%	28.2%	1.3%
% homo. recessives =	15.67%	3.52%	.24% = 19.4%

We would expect then, if crossing over is at random, to find 19.4 per cent of homozygous recessives in attached XXs at a point 62 units from the right end of the chromosome. The percentage of homozygosis found is significantly lower than expected. Sturtevant (1931) found 17.1 per cent homozygosis for scute, which is about 72 units from the right end of the X chromosome. This analysis was based on approximately 25,000 flies. Rhoades (1931) found about 18.6 per cent homozygosis for scute, but for ruby, which is about 64 units from the spindle fiber attachment, the percentage of homozygosis was found to be 17.7. Counts were made on about 42,000 flies. At a point 62 units from the spindle fiber the percentage of homozygosis recessives would be about 17.4 where 19.4 per cent would be expected on the basis of random crossing over.

Anderson's attached X data shows a similar discrepancy between the percentage of homozygosis found and the percentage expected on random crossing over. Chromosomes homozygous for the forked locus are assumed to have crossed over to the right of forked, and such chromatids are classed as crossovers in this region. Only one of the chromatids, equational at forked, is a crossover to the right of forked, but both are included to make up for the reciprocal crossovers to the right of forked which cannot be detected from the data. Considering these crossovers to the right of forked, there are 59.5 per cent of the chromatids with no crossovers, 37.2 per cent with one, and 3.3 per cent with two. In terms of crossover chiasmata, 25.6 per cent of the bivalent chromosomes have no chiasmata between the spindle fiber and end, 61.2 per cent have one chiasma, and 13.2 per cent have double chiasmata which break. If crossing over is at random the percentage of homozygosis for cut should be 16.5 per cent in the XX chromosomes listed in Anderson's table 6. The percentage of homozygosis actually found was 15.5, although for tan, still further to the right, the value was 16.1.

The percentage of homozygous recessives at the left end of attached X chromosomes is lower than expected if crossing over is at random. But, as Anderson and others have suggested, the

lower viability of the homozygous recessive segregates would reduce the percentage of these classes so that the true value might well approach the percentage expected on random assortment.

If there is any significant deficiency of homozygosis in attached X chromosomes, it would indicate that second cross-overs are not entirely at random. On the writer's hypothesis such a deficiency could be attributed to few half twists between the first and second cross over. For instance, if the average frequency of half twists were 0.25, the percentage of homozygous recessives 62 units from the fiber would be only 17.6 instead of 19.4 expected on random assortment. Belling's recent theory should always give random assortment for all cross overs. The modification of Belling's theory suggested by the writer would also fail to account for any deficiency of homozygous recessives. If crossing over is invariably at random between the four chromatids, Belling's theory would seem to be the most valid interpretation of the mechanism of crossing over. If, however, crossing over is not entirely at random, the writer's hypothesis seems to offer the only solution. Although there is a deficiency of homozygous segregates from attached X chromosomes, the nature of the genetic evidence does not justify any final conclusion concerning random assortment of the chromatids at the second cross over.

CHROMOSOME PAIRING AND CROSSING OVER.

When crossing over is eliminated in the *Drosophila* female there is also a loose association of homologous chromosomes. (Gowen 1922, 1928.) This behavior is undoubtedly analagous to the case of asynapsis in *Zea* (Beadle 1930). A decrease in crossing over may also be caused by inversions and translocations. Dr. Anderson informs me that his high non-disjunction line was caused by an inversion. In this case the decrease in crossing over was also associated with an increase in "non-disjunction" or failure of chromosome pairing. Dobzhansky (1931, 1932) has found that crossing over is decreased and non-disjunction increased in flies heterozygous for translocations. This behavior is attributed to conflicting attractions between homologous chromosome segments.

On either theory of crossing over the reduction or elimination of crossing over could be attributed to a differential rate of chromosome pairing compared with chromatid organization. At pachytene all four chromatids are associated, at diplotene only two chromatids can be associated and at telophase the two chromatids are united only at the spindle fiber constriction. If chromosome pairing is delayed then the sister chromatids might be at a stage commonly

found at diplotene, before the homologous chromosomes are paired, so that no chiasmata could be formed. In the cases of inversion no pairing of homologous genes would occur between inverted and normal chromosome segments and no crossovers could be produced in such regions. In heterozygous translocations chromosome pairing is delayed so that few chiasmata can be formed.

The genetic and cytological evidence shows that crossing over is most frequent at the distal end of the X chromosome (Painter, 1931) and at the distal ends of the third chromosome (Dobzhansky, 1930). This localization of crossing over would seem to indicate that chromosome pairing in *Drosophila* begins at the distal ends of the chromosomes and proceeds towards the spindle fiber. Crossing over would occur at the ends of the chromosomes because the chromatids are not sufficiently differentiated to prevent chiasma formation, but towards the spindle fiber the paired sister chromatids become so united that chiasma formation is no longer possible. Since chiasma formation is associated with crossing over, on either theory, there would be few crossovers in the spindle fiber region and frequent crossovers at the distal ends of the chromosomes.

Crossing over is increased in the X chromosome and in the autosomes of *Drosophila* by changes in temperature and by X-rays. (Plough 1917; Stern 1926; Muller 1925, 1926.) The increase in crossing over occurs primarily in the region of the spindle fiber attachment, but not in regions where crossing over is frequent in untreated flies. This behavior could be attributed to an acceleration of chromosome pairing so that in the region of the fiber attachment the chromosomes would be paired before the sister chromatids had sufficiently developed to prevent chiasma formation. Thus crossing over would be increased in the spindle fiber region of the chromosome.

Differences in types of chromosome association at meiosis might also be attributed to differences in the region where pairing begins. If pairing begins at the spindle fiber and proceeds slowly toward the ends, the chiasmata would be localized in the region of the fiber, as is the case in *Fritillaria* (Newton and Darlington, 1930). If pairing is completed before sister chromatids are sufficiently developed, then chiasmata will not be localized, but will be more or less uniformly distributed along the bivalent chromosome as is the case in *Lathyrus*, *Lilium* and *Vicia*. If pairing begins at the ends and proceeds slowly toward the middle, or if the sister chromatids develop rapidly, then the chiasmata will be terminal, as found in the Solanaceae.

Graubard (1932) has recently presented evidence, based on cross-

ing over in homologous chromosomes heterozygous for an inversion, which seems to indicate that pairing begins at the spindle fiber in the second chromosome of *Drosophila*.

SUMMARY.

A study of chromosome behavior at different stages of meiosis in a number of species of plants, together with an analysis of the genetic evidence in *Drosophila*, has provided some critical evidence in regard to the cytological mechanism of crossing over.

Janssen's partial chiasmatypy hypothesis has been supported by Darlington, Belling and Maeda, but it is shown that there is no critical evidence in favor of this theory. Belling has offered the only explanation of the mechanism of crossing over in connection with Janssen's hypothesis, but this explanation is not in accord with certain cytological and genetic evidence. Neither Belling's theory of crossing over nor Darlington's theory of meiosis can be reconciled with the cytological work of Robertson, Kaufmann and Sharp.

According to the writer's hypothesis crossing over is caused by breaks in two of the chromatids at a chiasma so that crossing over should be correlated with a reduction in the number of chiasmata between the diplotene and diakinesis stages of meiosis. In *Callisia repens* there is a considerable reduction in number of chiasmata per bivalent between the diplotene and metaphase stages. The work of other cytologists shows that the numbers of nodes or chiasmata are reduced during the prophases of meiosis in *Tulipa*, *Lilium*, *Primula*, *Rosa* and *Matthiola*.

The association of non-disjunction with a reduction in crossing over is apparently due to a weak association of homologous chromosomes in high non-disjunction lines of *Drosophila*. Where crossovers occur in the X chromosomes which pass to the same egg cell the partial chiasmatypy hypothesis would necessitate premature terminalization of chiasmata, in some cases for practically the entire crossover length of the X chromosome. On the writer's hypothesis non-disjunction with crossing over is attributed to the formation of few chiasmata all of which are broken before diakinesis, resulting in a weak association of homologous chromosomes.

The ratio of different types of second crossovers and the percentage of homozygosis in attached X chromosomes in *Drosophila* suggests that the second crossover may not be at random between any two of the four chromatids. If crossing over is not random at the second crossover the writer's hypothesis will account for the deficiency of homozygosis. It is impossible to account for these

genetic results on Belling's hypothesis or on any logical modification of his hypothesis.

Localization of chiasmata and crossovers is attributed to the type of chromosome pairing and to the relation between chromosome pairing and chromatid development.

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DESCRIPTION OF PLATE 44.

Meiosis in the pollen mother cells of Callisia repens. Magnification × 3000

Figure 1. Pachytene stage. No continuous spireme observed at any stage in meiosis.

Figure 2. Late pachytene. The six chromosomes can be observed more clearly at this stage. The nucleolus is always attached to one of the short bivalents.

Figure 3. Early diplotene showing the chromosomes forming the nodes and internodes.

Figure 4. Diplotene stage showing the number of nodes, most of which are probably chiasmata.

Figure 5. Early diakinesis showing the reduction in number of nodes or chiasmata compared with the diplotene stage.

Figures 6 and 7. Diakinesis. The nucleolus disappears between diakinesis and first metaphase.

Figure 8. Metaphase of the first meiotic division. The chromosomes show the coiled chromonemata.

Figure 9. Late anaphase showing spindle fiber attachment points. Two of the six chromosomes have a median attachment while four have terminal fiber attachments. Compare with text-figure 1.

Figure 10. The nucleolus seems to be attached to the the same chromosome in all cases. This chromosome at late diplotene is shown at the left. Typical diplotene stages are shown in the other five chromosomes.

Figure 11. The chromosome at the left is from a p. m. c. at early diakinesis. The other chromosomes show the number of chiasmata at diakinesis. Note the reduction in length of the bivalent from pachytene to diakinesis and the reduction in the number of nodes or chiasmata between diplotene and diakinesis.